Synthesis and DNA Polymerase α and β Inhibitory Activity of Alkyl *p*-Coumarates and Related Compounds

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***trans*- and *cis*-Icosyl, docosyl, and tetracosyl *p*-coumarates, constituents of *Artemisia annua* L., and their structurally-related compounds were synthesized and evaluated for inhibitory activity on DNA polymerases α and β. Among 30 compounds synthesized, octadecyl *trans*- and *cis*-*p*-coumarates and octadecyl *p*-hydroxyphenylpropiolate showed strong inhibitory activity on DNA polymerases α and β.**

Key words alkyl *p*-coumarate ester; DNA polymerase; inhibitory activity

A composite plant *Artemisia annua* L. is an annual herbaceous plant, which is known in China as a traditional antimalarial medicine, and in Southeast Asia as an antipyretic and hemostatic. Previous biological study on the active constituents of *A. annua* disclosed artemisinin, arteannuin B, and many sesquiterpenes as anti-malarial or anti-tumor components.[1)]

We have recently reinvestigated the chemical constituents of *A. annua* to isolate, along with novel sesquiterpenes, esters of *p*-coumaric acid with long-chain alcohols (**1**) as a mixture of six compounds of different chain length of C_{20}, C_{22}, C_{24}, and *cis* and *trans* isomers (Fig. 1).[2)]

An attempted examination of this mixture of alkyl *p*-coumarates showed inhibitory activity on DNA polymerases. On the basis of these results, we synthesized and biologically evaluated *p*-coumarates of different alkyl chain length and related compounds. The goal was to define the portions of the structure required for activity. In this paper, we describe chemical synthesis of **1** and its related compounds, and the inhibitory activity of these compounds on DNA polymerases α and β.

Results and Discussion

Synthesis of *p*-Coumarate and Related Compounds Direct esterification of carboxylic acid bearing phenolic hydroxy group with long-chain alcohols by Mistunobu reaction was reported by Appendino's group.[3)] According to this procedure, we synthesized *trans*-coumarate esters **1** of different chain length by Mitsunobu reactions of *p*-coumaric acid with the corresponding alcohols using diisopropyl azodicarboxylate (DIAD) and triphenylphosphine in tetrahydrofuran (THF) as shown in Chart 1. In this synthesis, we found that the reaction proceeds efficiently by adding a solution of DIAD in THF to the mixture of the alcohol, carboxylic acid, and triphenylphosphine in THF at room temperature, giving esters (*trans*-**1**, **3**—**9**) in good to excellent yields (Chart 1, Fig. 2).

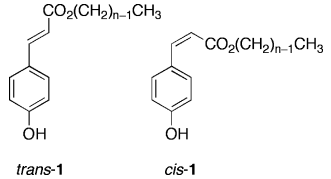
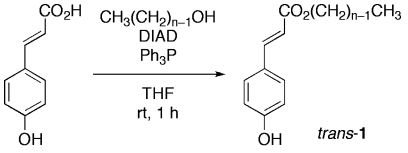


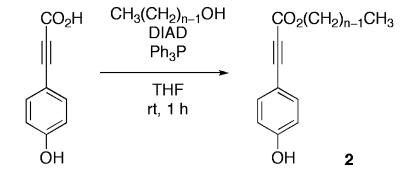
Fig. 1. *p*-Coumarate Esters Isolated from *Artemisia annua*
n=20, 22, and 24.

cis-Coumarate esters **1** were prepared by partial hydrogenation of propiolate esters **2** using the Lindlar catalyst and quinoline in ethyl acetate (Chart 2). In this reaction, ethyl acetate is the superior solvent to methanol; thus, hydrogenation in methanol resulted in over-reduction to give a saturated ester such as **12**. The propiolate esters **2** were prepared by Mitsunobu reactions of *p*-hydroxyphenylpropiolic acid[4)] with the corresponding alcohols.



n	10	12	14	16
yield (%)	85	85	93	88
n	18	20	22	24
yield (%)	94	92	88	89

Chart 1. Synthesis of *p*-Coumarate Esters by Mitsunobu Reaction



n	14	18	20	22	24
yield of **2** (%)	76	92	81	88	85
yield of *cis*-**1** (%)	89	91	87	84	82

Chart 2. Synthesis of Acetylenic-Derivatives and *cis*-Coumarate

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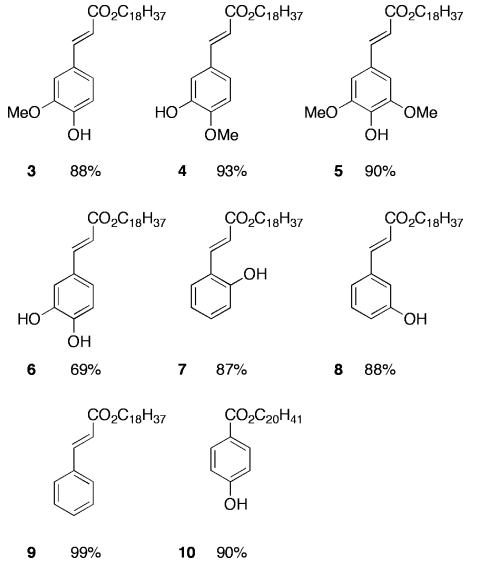

Fig. 2. Structures and Chemical Yields of Synthesized Analogous Compounds

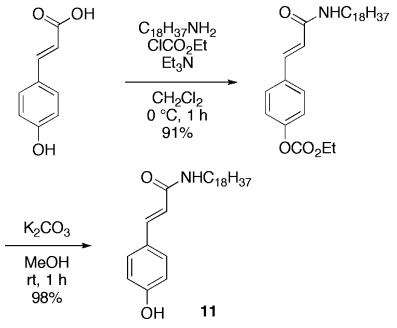

Chart 3. Synthesis of an Amide-Derivative

Ferulate **3**, isoferulate **4**, sinapate **5**, caffeate **6**, *o*-coumarate **7**, *m*-coumarate **8**, cinnamate **9**, and *p*-hydroxybenzoate **10** (Fig. 2) were prepared by Mitsunobu reaction using the procedure described above.

Amide **11** was prepared from *p*-coumaric acid and stearylamine *via* mixed anhydride with ethyl chloroformate, followed by alcoholysis of ethoxycarbonyl group (Chart 3).

Dihydrocoumarate **12** was prepared by hydrogenation of *trans*-**1** in the presence of palladium on carbon. *p*-Methoxy derivative **13** was prepared by methylation of *trans*-**1** ($n=18$) with methyl iodide (Chart 4).

Ketone **14** was prepared by aldol condensation of eicosan-2-one with *p*-methoxymethoxybenzaldehyde, followed by acid hydrolysis of the methoxymethyl protecting group (Chart 5).

In this synthesis, aldol condensation of eicosan-2-one with protected benzaldehyde took a long time to give a lower yield of the condensation product along with unreacted ketone; calculated yield based on recovered ketone was 53%.

Effect of the Synthesized Compounds on Mammalian DNA Polymerase Activity Selective inhibitors of mammalian DNA polymerases are considered a group of potentially useful cancer chemotherapy agents, because some DNA polymerase inhibitors suppress human cancer cell proliferation and have cytotoxicity.[5] Herein, the inhibitory activity of the synthesized compounds for calf DNA polymerase

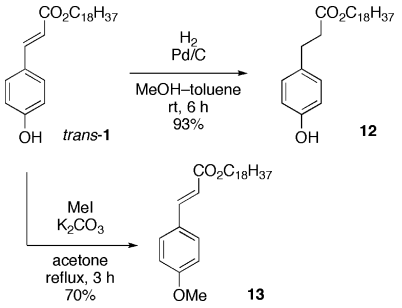

Chart 4. Synthesis of Dihydro- and *p*-Methoxy-Derivatives

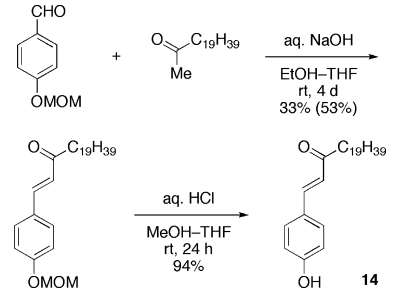

Chart 5. Synthesis of a Ketone-Derivative

α and rat DNA polymerase β, which are eukaryotic representative replicative and repair related DNA polymerases, respectively, was investigated.

As shown in Table 1, the inhibitory effects on DNA polymerase β activity by all compounds were stronger than those on DNA polymerase α. In *trans*-coumarate esters, the inhibitory effect of the compound bearing C_{18}-alkyl chain (*i.e.*, **1** ($n=18$)) was strongest in synthesized compounds, and the order of DNA polymerase inhibitory effects was as follows: $n=18>n=20>n=16>n=22>n=14$ and $n=24$. These results suggest that the chain length at the alcohol moiety of compound **1** must be very important for the inhibition of DNA polymerase activity. In the configuration of the compound (*i.e.*, *cis*-**1** and *trans*-**1**), *cis*-**1** was a stronger and weaker inhibitor than *trans*-**1** against DNA polymerases α and β; respectively, therefore, the configuration of the double bond is important for inhibition. Compound **2**, which is the acetylene form of compound **1**, also inhibited the activity of mammalian DNA polymerases α and β. The inhibitory effect of compound **2** showed the same tendency and was strong as that of compound **1**, and in order of their effect, the alkyl chain length of compound **2** ranked as follows: $n=18>n=20>n=22>n=14>n=24$. Next, we focused the substitution pattern on the benzene ring of **1** and the character of carbonyl group of **1**. We synthesized compounds **3** to **14** having different substituents on the phenyl group and different carbonyl character (*i.e.*, amide, ketone), and the inhibitory activity on DNA polymerases α and β was investigated. Compounds **3**—**14** had no effect on the activity, suggesting that both *p*-hydroxyphenyl group and ester carbonyl group of compounds **1** and **2** are essential for the inhibition of DNA polymerases. These moieties of compounds **1** and **2** may directly bind to the active site of DNA polymerases α and β and inhibit the activities, because these compounds did not intercalate to double-stranded DNA as a template-primer.[6]

By continuing the analysis of the relationship between

Table 1. IC_{50} Values of the Synthesized Compounds on the Activities of Mammalian DNA Polymerases α and β

Compound	IC_{50} values (μM)	
	Calf DNA polymerase α	Rat DNA polymerase β
trans-**1** (n=10)	>1000	>1000
trans-**1** (n=12)	>1000	>1000
trans-**1** (n=14)	>1000	>1000
trans-**1** (n=16)	382±15	145±7.2
trans-**1** (n=18)	68.0±3.2	23.1±1.1
trans-**1** (n=20)	334±13	122±6.0
trans-**1** (n=22)	>1000	359±18
trans-**1** (n=24)	>1000	>1000
cis-**1** (n=14)	>1000	185±9.1
cis-**1** (n=18)	45.5±2.0	32.1±1.5
cis-**1** (n=20)	271±12	158±7.8
cis-**1** (n=22)	>1000	392±16
cis-**1** (n=24)	>1000	>1000
2 (n=14)	>1000	386±17
2 (n=18)	53.0±2.6	16.3±0.8
2 (n=20)	192±9.5	71.2±3.6
2 (n=22)	304±14	221±11
2 (n=24)	>1000	>1000
3	>1000	>1000
4	>1000	>1000
5	>1000	>1000
6	>1000	>1000
7	>1000	>1000
8	>1000	>1000
9	>1000	>1000
10	>1000	>1000
11	>1000	>1000
12	>1000	>1000
13	>1000	>1000
14	>1000	>1000

These compounds were incubated with each DNA polymerase (0.05 units). One unit of DNA polymerase activity was defined as the amount of enzyme that catalyzed the incorporation of 1 nmol of dNTP (dTTP) into the synthetic DNA template-primers (*i.e.*, poly(dA)/oligo$(dT)_{12-18}$, A/T=2/1) in 60 min at 37 °C under normal reaction conditions for each enzyme (refs. 9, 10). Enzyme activity in the absence of the compound was taken as 100%. Data are expressed as the mean±S.D.; n=3.

structure and function, more effective DNA polymerase inhibitors than compounds **1** and **2** (n=18) will be discovered to explore useful anti-cancer agents in the future.

Experimental

All melting points were recorded on Yanagimoto hot plate melting points apparatus and are uncorrected. IR spectra were taken by Shimadzu FTIR-8200 spectrophotometer. NMR spectra were taken by Varian Mercury 300 spectrometer at 300 MHz for ^{1}H- and 75 MHz for ^{13}C-NMR. MS and HR-MS spectra were taken by Hitachi M-4000 spectrometer.

Octadecyl *p*-Coumarate: *trnas*-1 (n=18); General Procedure for the Esterification by Mitsunobu Reaction To a solution of octadecanol (270 mg, 1 mmol), *p*-coumaric acid (328 mg, 2 mmol), and triphenylphosphine (524 mg, 2 mmol) in THF (7 ml) was added a solution of diethyl azodicarboxylate (0.38 ml, 2 mmol) in THF (3 ml) at room temperature (rt) over 15 min period, and the whole was stirred for 1 h at rt. The reaction mixture was diluted with ethyl acetate, and was washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate=9/1) to give 393 mg (94%) of white solid. mp 84—87 °C. IR (Nujol) cm^{-1}: 3380, 1674, 1602, 1585. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (30H, m), 1.70 (2H, quintet, J=6.9 Hz), 4.20 (2H, t, J=6.9 Hz), 6.00 (1H, s), 6.30 (1H, d, J=15.9 Hz), 6.86 (2H, d, J=6.9 Hz), 7.42 (2H, d, J=6.9 Hz), 7.63 (1H, d, J=15.9 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 26.0, 28.7, 29.3—29.7 (29.3, 29.4, 29.5, 29.58, 29.65, 29.69), 31.9, 64.8, 115.5, 115.9, 127.1, 130.0, 144.6, 157.9, 167.9. MS (EI) *m/z*: 416 (M^+), 164. HR-MS (EI) *m/z*: 416.3279 (Calcd for $C_{27}H_{44}O_3$: 416.3290).

Decyl *p*-Coumarate: *trans*-1 (n=10) mp 62—64 °C. IR (Nujol) cm^{-1}: 3379, 1672, 1603, 1585. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (14H, m), 1.70 (2H, quintet, J=6.9 Hz), 4.20 (2H, t, J=6.9 Hz), 6.30 (1H, d, J=15.9 Hz), 6.54 (1H, br s), 6.87 (2H, d, J=6.6 Hz), 7.42 (2H, d, J=6.6 Hz), 7.63 (1H, d, J=15.9 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.6, 25.9, 28.7, 29.2, 29.3, 29.5 (×2), 31.9, 64.9, 115.2, 115.9, 126.9, 130.0, 144.8, 158.1, 168.2. MS (EI) *m/z*: 304 (M^+), 164. HR-MS (EI) *m/z*: 304.2048 (Calcd for $C_{19}H_{28}O_3$: 304.2038).

Dodecyl *p*-Coumarate: *trans*-1 (n=12) mp 73—75 °C. IR (Nujol) cm^{-1}: 3377, 1674, 1602, 1585. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H , t, J=6.9 Hz), 1.2—1.5 (18H, m), 1.70 (2H, quintet, J=6.9 Hz), 4.20 (2H, t, J=6.9 Hz), 6.30 (1H, d, J=16.2 Hz), 6.33 (1H, s), 6.82 (2H, d, J=9.0 Hz), 7.41 (2H, d, J=9.0 Hz), 7.63 (1H, d, J=16.2 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 25.9, 28.7, 29.3—29.6 (29.25, 29.32, 29.50, 29.56, 29.61), 31.9, 64.9, 115.3, 115.9, 127.0, 130.0, 144.7, 158.1, 168.1. MS (EI) *m/z*: 332 (M^+), 164. HR-MS (EI) *m/z*: 332.2341 (Calcd for $C_{21}H_{32}O_3$: 332.2351).

Tetradecyl *p*-Coumarate: *trans*-1 (n=14) mp 75—78 °C. IR (Nujol) cm^{-1}: 3380, 1674, 1603, 1585. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (22H, m), 1.70 (2H, quintet, J=6.9 Hz), 4.20 (2H, t, J=6.9 Hz), 6.03 (1H, s), 6.30 (1H, d, J=15.9 Hz), 6.85 (1H, d, J=6.6 Hz), 7.42 (1H, d, J=6.6 Hz), 7.63 (1H, d, J=15.9 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 26.0, 28.7, 29.3—29.7 (29.28, 29.34, 29.52, 29.57, 29.63, 29.66), 31.9, 64.8, 115.5, 115.9, 127.1, 130.0, 144.6, 157.9, 167.9. MS (EI) *m/z*: 360 (M^+), 164. HR-MS (EI) *m/z*: 360.2682 (Calcd for $C_{23}H_{36}O_3$: 360.2664).

Hexadecyl *p*-Coumarate: *trans*-1 (n=16) mp 82—84 °C. IR (Nujol) cm^{-1}: 3380, 1674, 1602, 1585. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.6 Hz), 1.2—1.5 (26H, m), 1.70 (2H, quintet, J=6.6 Hz), 4.20 (2H, t, J=6.6 Hz), 6.23 (1H, s), 6.30 (1H, d, J=15.9 Hz), 6.86 (2H, d, J=6.6 Hz), 7.42 (2H, d, J=6.6 Hz), 7.63 (1H, d, J=15.9 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 26.0, 28.7, 29.3—29.7 (29.28, 29.34, 29.52, 29.58, 29.64, 29.68), 31.9, 64.9, 115.4, 115.9, 127.0, 130.0, 144.7, 158.0, 168.0. MS (EI) *m/z*: 388 (M^+), 164. HR-MS (EI) *m/z*: 388.2969 (Calcd for $C_{25}H_{40}O_3$: 388.2977).

Icosyl *p*-Coumarate: *trans*-1 (n=20) mp 89—90 °C. IR (Nujol) cm^{-1}: 3380, 1675, 1601, 1585. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (34H, m), 1.70 (2H, quintet, J=6.9 Hz), 4.20 (2H, t, J=6.9 Hz), 6.00 (1H, s), 6.30 (1H, d, J=16.8 Hz), 6.85 (2H, d, J=6.9 Hz), 7.42 (2H, d, J=6.9 Hz), 7.63 (1H, d, J=16.8 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 26.0, 28.7, 29.3—29.7 (29.28, 29.35, 29.53, 29.58, 29.65, 29.69), 31.9, 64.8, 115.5, 115.9, 127.1, 130.0, 144.5, 157.9, 167.9. MS (EI) *m/z*: 444 (M^+), 164. HR-MS (EI) *m/z*: 444.3621 (Calcd for $C_{29}H_{48}O_3$: 444.3603).

Docosyl *p*-Coumarate: *trans*-1 (n=22) mp 88—91 °C. IR (Nujol) cm^{-1}: 3380, 1675, 1599 1585. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (38H, m), 1.71 (2H, quintet, J=6.9 Hz), 4.19 (2H, t, J=6.9 Hz), 5.76 (1H, s), 6.30 (1H, d, J=15.9 Hz), 6.85 (2H, d, J=8.7 Hz), 7.43 (2H, d, J=8.7 Hz), 7.63 (1H, d, J=15.9 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 26.0, 28.7, 29.28, 29.3—29.7 (29.34, 29.53, 29.58, 29.65, 29.69), 31.9, 64.8, 115.6, 115.9, 127.2, 129.9, 144.5, 157.8, 167.8. MS (EI) *m/z*: 472 (M^+), 164. HR-MS (EI) *m/z*: 472.3921 (Calcd for $C_{31}H_{52}O_3$: 472.3916).

Tetracosyl *p*-Coumarate: *trans*-1 (n=24) mp 95—96 °C. IR (Nujol) cm^{-1}: 3381, 1674, 1602, 1585. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (42H, m), 1.70 (2H, quintet, J=6.9 Hz), 4.19 (2H, t, J=6.9 Hz), 5.88 (1H, s), 6.30 (1H, d, J=15.9 Hz), 6.85 (2H, d, J=6.6 Hz), 7.42 (2H, d, J=6.6 Hz), 7.68 (1H, d, J=15.9 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 26.0, 28.7, 29.3—29.7 (29.28, 29.35, 29.53, 29.65, 29.69), 31.9, 64.8, 115.5, 115.9, 127.1, 129.9, 144.5, 157.8, 167.8. MS (EI) *m/z*: 500 (M^+), 164. HR-MS (EI) *m/z*: 500.4242 (Calcd for $C_{33}H_{56}O_3$: 500.4229).

Octadecyl *cis-p*-Coumarate: *cis*-1 (n=18); General Procedure for the Partial Hydrogenation of the Acetylene A mixture of the acetylene **2** of n=18 (83 mg, 0.2 mmol), Lindlar catalyst (5% palladium on calcium carbonate poisoned with lead, 20 mg), and quinoline (0.071 ml, 0.6 mmol) in ethyl acetate (2 ml) was stirred under hydrogen atmosphere for 10 min at rt. The reaction mixture was filtered, and the filtrate was washed with 10% aqueous hydrochloric acid and brine, dried over sodium sulfate, filtered, and concentrated. The residue was recrystallized from hexane to give 76 mg (92%) of white solid. mp 72—74 °C. IR (Nujol) cm^{-1}: 3368, 1699, 1607. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (30H, m), 1.65 (2H, quintet, J=6.9 Hz), 4.13 (2H, t, J=6.9 Hz), 5.83 (1H, d, J=12.9 Hz), 6.07 (1H, s), 6.77 (2H, d, J=6.9 Hz), 6.86 (1H, d, J=12.9 Hz), 7.59 (2H, d, J=6.9 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 26.0, 28.5, 29.3—29.7 (29.26, 29.35, 29.51, 29.57, 29.65, 29.69), 31.9, 64.7, 115.1, 116.9, 127.2, 132.2, 143.9, 157.0, 167.2. MS (EI) *m/z*: 416 (M^+), 164. HR-MS (EI) *m/z*: 416.3300 (Calcd for $C_{27}H_{44}O_3$: 416.3290).

Tetradecyl *cis-p*-Coumarate: *cis*-1 (n=14) mp 60—62 °C. IR (Nujol) cm^{-1}: 3371, 1699, 1607. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—

1.5 (22H, m), 1.65 (2H, quintet, J=6.9 Hz), 4.13 (2H, t, J=6.9 Hz), 5.83 (1H, d, J=12.6 Hz), 6.10 (1H, br), 6.77 (2H, d, J=6.9 Hz), 6.86 (1H, d, J=12.6 Hz), 7.59 (2H, d, J=6.9 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 26.0, 28.5, 29.3—29.6 (29.25, 29.34, 29.50, 29.57, 29.64), 31.9, 64.7, 115.1, 116.9, 127.1, 132.2, 144.0, 157.0, 167.2. MS (EI) *m/z*: 360 (M^+), 164. HR-MS (EI) *m/z*: 360.2682 (Calcd for $C_{23}H_{36}O_3$: 360.2664).

Icosyl *cis*-*p*-Coumarate: *cis*-1 (*n*=20) mp 74—75 °C. IR (Nujol) cm^{-1}: 3383, 1712, 1686, 1601. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (34H, m), 1.65 (2H, quintet, J=6.9 Hz), 4.13 (2H, t, J=6.9 Hz), 5.83 (1H, d, J=12.6 Hz), 6.10 (1H, br), 6.77 (2H, d, J=6.9 Hz), 6.86 (1H, d, J=12.6 Hz), 7.59 (2H, d, J=6.9 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 25.9, 28.5, 29.3—29.7 (29.25, 29.34, 29.50, 29.57, 29.64, 29.69), 31.9, 64.7, 115.1, 116.9, 127.1, 132.2, 144.0, 157.1, 167.2. MS (EI) *m/z*: 444 (M^+), 164. HR-MS (EI) *m/z*: 444.3623 (Calcd for $C_{29}H_{48}O_3$: 444.3603).

Docosyl *cis*-*p*-Coumarate: *cis*-1 (*n*=22) mp 76—77 °C. IR (Nujol) cm^{-1}: 3383, 1713, 1686, 1603. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (38H, m), 1.65 (2H, quintet, J=6.9 Hz), 4.13 (2H, t, J=6.9 Hz), 5.83 (1H, d, J=12.6 Hz), 6.07 (1H, br s), 6.78 (2H, d, J=6.9 Hz), 6.86 (1H, d, J=12.6 Hz), 7.59 (2H, d, J=6.9 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 26.0, 28.6, 29.3—29.7 (29.26, 29.35, 29.52, 29.57, 29.65, 29.69), 31.9, 64.7, 115.1, 116.9, 127.2, 132.2, 143.9, 157.0, 167.2. MS (EI) *m/z*: 472 (M^+), 164. HR-MS (EI) *m/z*: 472.3940 (Calcd for $C_{31}H_{52}O_3$: 472.3916).

Tetracosyl *cis*-*p*-Coumarate: *cis*-1 (*n*=24) mp 81—83 °C. IR (Nujol) cm^{-1}: 3385, 1713, 1686, 1601. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (42H, m), 1.65 (2H, quintet, J=6.9 Hz), 4.13 (2H, t, J=6.9 Hz), 5.83 (1H, d, J=12.6 Hz), 6.09 (1H, br s), 6.78 (2H, d, J=6.9 Hz), 6.86 (1H, d, J=12.6 Hz), 7.59 (2H, d, J=6.9 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 26.0, 28.6, 29.3—29.7 (29.26, 29.35, 29.52, 29.57, 29.65, 29.69), 31.9, 64.7, 115.1, 116.9, 127.2, 132.2, 143.9, 157.0, 167.1. MS (EI) *m/z*: 500 (M^+), 164. HR-MS (EI) *m/z*: 500.4238 (Calcd for $C_{33}H_{56}O_3$: 500.4229).

Octadecyl *p*-Hydroxyphenylpropiolate: 2 (*n*=18) By the same procedure (Mitsunobu reaction) for preparation of *p*-coumarate ester, the title compound was prepared from *p*-hydroxyphenylpropiolic acid and octadecanol in 91% yield after purification by silica gel column chromatography eluting with hexane/ethyl acetate=9/1. mp 87—88 °C. IR (Nujol) cm^{-1}: 3219, 2206, 1659, 1602. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (30H, m), 1.71 (2H, quintet, J=6.9 Hz), 4.23 (2H, t, J=6.9 Hz), 5,95 (1H, s), 6.85 (2H, d, J=6.9 Hz), 7.45 (2H, d, J=6.9 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 25.8, 28.5, 29.2—29.7 (29.20, 29.34, 29.47, 29.56, 29.68), 31.9, 66.4, 80.0, 87.4, 111.3, 115.9, 135.2, 154.9, 158.1. MS (EI) *m/z*: 414 (M^+), 165. HR-MS (EI) *m/z*: 414.3130 (Calcd for $C_{27}H_{42}O_3$: 414.3134).

Tetradecyl *p*-Hydroxyphenylpropiolate: 2 (*n*=14) mp 72—74 °C. IR (Nujol) cm^{-1}: 3219, 2206, 1660, 1605. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (22H, m), 1.71 (2H, quintet, J=6.9 Hz), 4.23 (2H, t, J=6.9 Hz), 6.10 (1H, br), 6.84 (2H, d, J=6.9 Hz), 7.46 (2H, d, J=6.9 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 25.8, 28.4, 29.2—29.7 (29.19, 29.33, 29.46, 29.54, 29.63, 29.66), 31.9, 66.4, 79.9, 87.5, 111.2, 115.9, 135.2, 155.0, 158.2. MS (EI) *m/z*: 358 (M^+), 164. HR-MS (EI) *m/z*: 358.2516 (Calcd for $C_{23}H_{34}O_3$: 358.2508).

Icosyl *p*-Hydroxyphenylpropiolate: 2 (*n*=20) mp 92—93 °C. IR (Nujol) cm^{-1}: 3223, 2206, 1661, 1605. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (34H, m), 1.73 (2H, quintet, J=6.9 Hz), 4.23 (2H, t, J=6.9 Hz), 6.02 (1H, s), 6.84 (2H, d, J=6.9 Hz), 7.46 (2H, d, J=6.9 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 25.8, 28.5, 29.2—29.7 (29.21, 29.35, 29.48, 29.57, 29.69), 31.9, 66.4, 80.0, 87.5, 111.3, 115.9, 135.2, 155.0, 158.1. MS (EI) *m/z*: 442 (M^+), 164. HR-MS (EI) *m/z*: 442.3452 (Calcd for $C_{29}H_{46}O_3$: 442.3447).

Docosyl *p*-Hydroxyphenylpropiolate: 2 (*n*=22) mp 95—96 °C. IR (Nujol) cm^{-1}: 3223, 2206, 1661, 1605. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (38H, m), 1.71 (2H, quintet, J=6.9 Hz), 4.22 (2H, t, J=6.9 Hz), 6.03 (1H, s), 6.84 (2H, d, J=6.6 Hz), 7.46 (2H, d, J=6.6 Hz). ^{13}C-NMR ($CDCl_3$: DMSO-d_6=10 : 1) δ: 13.8, 22.4, 25.5, 28.2, 28.9, 29.0—29.4 (29.03, 29.18, 29.25, 29.37), 31.6, 65.7, 79.5, 87.5, 109.3, 115.8, 134.7, 154.3, 159.8. MS (EI) *m/z*: 470 (M^+), 164. HR-MS (EI) *m/z*: 470.3761 (Calcd for $C_{31}H_{50}O_3$: 470.3760).

Tetracosyl *p*-Hydroxyphenylpropiolate: 2 (*n*=24) mp 98—99 °C. IR (Nujol) cm^{-1}: 3223, 2208, 1661, 1605. ^{1}H-NMR ($CDCl_3$: DMSO-d_6=10 : 1) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (42H, m), 1.70 (2H, quintet, J=6.9 Hz), 4.20 (2H, t, J=6.9 Hz), 6.83 (2H, d, J=6.9 Hz), 7.44 (2H, d, J=6.9 Hz), 9.38 (1H, s). ^{13}C-NMR ($CDCl_3$: DMSO-d_6=10 : 1) δ: 14.0, 22.5, 25.7, 28.3, 29.1—29.5 (29.06, 29.18, 29.32, 29.40, 29.51), 31.7, 65.8, 79.6, 87.7, 109.6, 115.9, 134.9, 154.5, 159.8. MS (EI) *m/z*: 498 (M^+), 164. HR-MS (EI) *m/z*: 498.4069 (Calcd for $C_{33}H_{54}O_3$: 498.4073).

Octadecyl Ferulate (3) According to the general procedure for Mitsunobu reaction described above, the title compound was prepared from ferulic acid and octadecanol. mp 65—66 °C. IR (Nujol) cm^{-1}: 3550, 1682, 1622, 1597. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.6 Hz), 1.2—1.5 (30H, m), 1.70 (2H, quintet, J=6.9 Hz), 3.92 (3H, s), 4.19 (2H, t, J=6.9 Hz), 5.92 (1H, s), 6.29 (1H, d, J=15.9 Hz), 6.91 (1H, d, J=7.8 Hz), 7.03 (1H, d, J=1.8 Hz), 7.07 (1H, dd, J=1.8, 7.8 Hz), 7.61 (1H, d, J=15.9 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 26.0, 28.8, 29.3—29.7 (29.28, 29.34, 29.52, 29.57, 29.63, 29.67), 31.9, 55.9, 64.6, 109.3, 114.7, 115.6, 123.0, 127.1, 144.6, 146.7, 147.9, 167.4. MS (EI) *m/z*: 446 (M^+), 194. HR-MS (EI) *m/z*: 446.3412 (Calcd for $C_{28}H_{46}O_4$: 446.3396).

Octadecyl Isoferulate (4) mp 68—69 °C. IR (Nujol) cm^{-1}: 3566, 1697, 1631, 1614, 1583. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (30H, m), 1.69 (2H, quintet, J=6.9 Hz), 3.92 (3H, s), 4.18 (2H, t, J=6.9 Hz), 5.70 (1H, s), 6.29 (1H, d, J=15.9 Hz), 6.83 (1H, d, J=8.4 Hz), 7.03 (1H, dd, J=2.1, 8.4 Hz), 7.14 (1H, d, J=2.1 Hz), 7.58 (1H, d, J=15.9 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 26.0, 28.7, 29.3—29.7 (29.28, 29.34, 29.52, 29.57, 29.64, 29.68), 31.9, 56.0, 64.6, 110.5, 113.0, 116.3, 121.7, 128.1, 144.3, 145.8, 148.4, 167.4. MS (EI) *m/z*: 446 (M^+), 194. HR-MS (EI) *m/z*: 446.3413 (Calcd for $C_{28}H_{46}O_4$: 446.3396).

Octadecyl Sinapate (5) mp 72—73 °C. IR (Nujol) cm^{-1}: 3521, 1707, 1636, 1609. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (30H, m), 1.70 (2H, quintet, J=6.9 Hz), 3.92 (6H, s), 4.19 (2H, t, J=6.9 Hz), 5.78 (1H, s), 6.31 (1H, d, J=15.9 Hz), 6.77 (2H, s), 7.59 (1H, d, J=15.9 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 26.0, 28.8, 29.3—29.7 (29.28, 29.33, 29.53, 29.58, 29.63, 29.67), 31.9, 56.3, 64.6, 105.0, 116.0, 125.9, 137.0, 144.8, 147.2, 167.2. MS (EI) *m/z*: 476 (M^+), 180. HR-MS (EI) *m/z*: 476.3520 (Calcd for $C_{29}H_{48}O_5$: 476.3502).

Octadecyl Caffeate (6) mp 107—108 °C. IR (Nujol) cm^{-1}: 3479, 3308, 1682, 1607. ^{1}H-NMR ($CDCl_3$: DMSO-d_6=10 : 1) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (30H, m), 1.69 (2H, quintet, J=6.9 Hz), 4.16 (2H, t, J=6.9 Hz), 6.21 (1H, d, J=15.9 Hz), 6.84 (1H, d, J=8.1 Hz), 6.91 (1H, dd, J=2.1, 8.1 Hz), 7.07 (1H, d, J=2.1 Hz), 7.53 (1H, d, J=15.9 Hz), 8.37 (2H, br). ^{13}C-NMR ($CDCl_3$: DMSO-d_6=10 : 1) δ: 13.6, 22.1, 25.4, 28.2, 28.8—29.1 (28.75, 28.79, 28.99, 29.03, 29.09, 29.12), 31.4, 63.9, 113.9, 114.3, 115.2, 121.1, 126.1, 144.5, 144.7, 147.2, 167.0. MS (EI) *m/z*: 432 (M^+), 180. HR-MS (EI) *m/z*: 432.3254 (Calcd for $C_{27}H_{44}O_4$: 432.3240).

Octadecyl *o*-Coumarate (7) mp 83—85 °C. IR (Nujol) cm^{-1}: 3196, 1670, 1601. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (30H, m), 1.71 (2H, quintet, J=6.9 Hz), 4.23 (2H, t, J=6.9 Hz), 6.66 (1H, d, J=16.2 Hz), 6.88 (1H, ddd, J=1.2, 7.5, 7.5 Hz), 6.92 (1H, dd, J=1.2, 7.5 Hz), 7.01 (1H, s), 7.23 (1H, ddd, J=1.2, 7.5, 7.5 Hz), 7.47 (1H, dd, J=1.2, 7.5 Hz), 8.06 (1H, d, J=16.2 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 26.0, 28.7, 29.3—29.7 (29.28, 29.34, 29.53, 29.58, 29.65, 29.69), 31.9, 65.0, 116.4, 118.3, 120.6, 121.7, 129.2, 131.4, 140.7, 155.5, 168.7. MS (EI) *m/z*: 416 (M^+), 146. HR-MS (EI) *m/z*: 416.3303 (Calcd for $C_{27}H_{44}O_3$: 416.3290).

Octadecyl *m*-Coumarate (8) mp 78—79 °C. IR (Nujol) cm^{-1}: 3379, 1688, 1641, 1595. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (30H, m), 1.70 (2H, quintet, J=6.9 Hz), 4.21 (2H, t, J=6.9 Hz), 6.00 (1H, br), 6.41 (1H, d, J=15.9 Hz), 6.89 (1H, m), 7.04 (1H, m), 7.09 (1H, m), 7.25 (1H, t, J=7.8 Hz), 7.63 (1H, d, J=15.9 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 26.0, 28.7, 29.3—29.7 (29.28, 29.35, 29.53, 29.58, 29.65, 29.69), 31.9, 65.0, 114.6, 117.5, 118.4, 120.6, 130.1, 135.9, 144.7, 156.3, 167.6. MS (EI) *m/z*: 416 (M^+), 164. HR-MS (EI) *m/z*: 416.3287 (Calcd for $C_{27}H_{44}O_3$: 416.3290.).

Octadecyl Cinnamate (9) mp 45—46 °C. IR (Nujol) cm^{-1}: 1713, 1639, 1580. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (30H, m), 1.70 (2H, quintet, J=6.9 Hz), 4.20 (2H, t, J=6.9 Hz), 6.44 (1H, d, J=16.2 Hz), 7.36—7.39 (3H, m), 7.53 (2H, m), 7.68 (1H, d, J=16.2 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 26.0, 28.7, 29.3—29.7 (29.28, 29.35, 29.53, 29.58, 29.65, 29.69), 31.9, 64.7, 118.3, 128.0, 128.8, 130.2, 134.5, 144.5, 167.1. MS (EI) *m/z*: 400 (M^+), 148. HR-MS (EI) *m/z*: 400.3351 (Calcd for $C_{27}H_{44}O_2$: 400.3341).

Icosyl *p*-Hydroxybenzoate (10) mp 70—72 °C. IR (Nujol) cm^{-1}: 3396, 1688, 1605, 1587. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (34H, m), 1.75 (2H, quintet. J=6.9 Hz), 4.29 (2H, t, J=6.9 Hz), 6.48 (1H, s), 6.88 (2H, d, J=6.9 Hz), 7.95 (2H, d, J=6.9 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 26.1, 28.7, 29.3—29.7 (29.29, 29.35, 29.53, 29.59, 29.65, 29.69), 31.9, 65.1, 115.2, 122.7, 131.9, 160.2, 167.0. MS (EI) *m/z*: 418 (M^+), 138. HR-MS (EI) *m/z*: 418.3448 (Calcd for $C_{27}H_{46}O_3$: 418.3447).

***N*-Octadecyl-*p*-coumaramide (11)** To a solution of *p*-coumaric acid (394 mg, 2.4 mmol) in dichloromethane (10 ml) were added triethylamine (0.67 ml, 4.8 mmol) and ethyl chloroformate (0.46 ml, 4.8 mmol) at 0 °C,

and the mixture was stirred for 0.5 h at the same temperature. Stearylamine (539 mg, 2 mmol) was added, and the whole was stirred for 1 h at 0 °C. 10% aqueous sodium hydroxide solution was added, and the mixture was extracted with dichloromethane. Combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by silica gel column chromatography (chloroform/ethyl acetate=20/1) to give 888 mg (91%) of *O*-ethoxycarbonyl-*N*-octadecyl-*p*-coumaramide as a white solid. mp 96—97 °C. IR (Nujol) cm^{-1}: 3323, 1759, 1749, 1651, 1614. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (30H, m), 1.39 (3H, t, J=7.2 Hz), 1.56 (2H, quintet, J=6.9 Hz), 3.37 (2H, q, J=6.9 Hz), 4.33 (2H, q, J=7.2 Hz), 5.80 (1H, br), 6.34 (1H, d, J=15.6 Hz), 7.17 (2H, d, J=7.8 Hz), 7.49 (2H, d, J=7.8 Hz), 7.59 (1H, d, J=15.6 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 14.2, 22.7, 27.0, 29.3—29.7 (29.32, 29.53, 29.57, 29.63. 29.67), 31.9, 39.8, 65.0, 121.2, 121.4, 128.8, 132.8, 139.5, 151.8, 153.3, 165.6. MS (EI) *m*/*z*: 487 (M^+), 147. HR-MS (EI) *m*/*z*: 487.3641 (Calcd for $C_{30}H_{49}NO_4$: 487.3662).

To a suspension of the carbonate (731 mg, 1.5 mmol) in methanol (20 ml) was added potassium carbonate (210 mg, 1.5 mmol) at rt. The whole was stirred for 1 h at the same temperature. Ten percent hydrochloric acid was added at 0 °C, and the mixture was extracted with dichloromethane. Combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by silica gel column chromatography (chloroform/ethyl acetate=4/1) to give 613 mg (98%) of **11** as a white solid. mp 108–110 °C. IR (Nujol) cm^{-1}: 3319, 1651, 1614. ^{1}H-NMR ($CDCl_3$: DMSO-d_6=10 : 1) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (30H, m), 1.55 (2H, quintet, J=6.5 Hz), 3.33 (2H, q, J=6.5 Hz), 6.31 (1H, d, J=15.9 Hz), 6.54 (1H, t, J=6.5 Hz), 6.84 (2H, d, J=8.1 Hz), 7.35 (2H, d, J=8.1 Hz), 7.51 (1H, d, J=15.9 Hz), 9.20 (1H, s). ^{13}C-NMR ($CDCl_3$: DMSO-d_6=10 : 1) δ: 13.8, 22.3, 26.7, 29.0—29.3 (28.99, 29.02, 29.23, 29.33), 31.6, 39.3, 115.6, 117.7, 126.1, 129.0, 139.9, 158.6, 166.3. MS (EI) *m*/*z*: 416 (MH^+), 147. HR-MS (EI) *m*/*z*: 415.3449 (Calcd for $C_{27}H_{45}NO_2$: 415.3450).

Octadecyl *p*-Dihydrocoumarate (12) A mixture of octadecyl *p*-coumarate (83 mg, 0.2 mmol) and palladium on carbon (10%, 8 mg) in a mixture of methanol (10 ml) and toluene (5 ml) was stirred under hydrogen atmosphere for 6 h at rt. The reaction mixture was filtered, and the filtrate was concentrated and purified by silica gel column chromatography (hexane/ethyl acetate=9/1) to give 78 mg (93%) of a white solid. mp 49—50 °C. IR (Nujol) cm^{-1}: 3332, 1734, 1724, 1616, 1602. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (30H, m), 1.59 (2H, quintet, J=6.9 Hz), 2.59 (2H, t, J=8.2 Hz), 2.88 (2H, t, J=8.2 Hz), 4.06 (2H, t, J=6.9 Hz), 5.32 (1H, s), 6.74 (2H, d, J=6.6 Hz), 7.05 (2H, d, J=6.6 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 25.9, 28.6, 29.2—29.7 (29.23, 29.34, 29.50, 29.57, 29.64, 29.68), 30.1, 31.9, 36.3, 64.8, 115.3, 129.4, 132.5, 154.1, 173.5. MS (EI) *m*/*z*: 418 (M^+), 224. HR-MS (EI) *m*/*z*: 418.3452 (Calcd for $C_{27}H_{46}O_3$: 418.3447).

Octadecyl *O*-Methyl-*p*-coumarate (13) To a solution of the phenol (83 mg, 0.2 mmol) in *N*,*N*-dimethylformamide (2 ml) were added potassium carbonate (41 mg, 0.3 mmol) and methyl iodide (0.02 ml, 0.3 mmol), and the mixture was stirred for 2 h at rt. Water was added, and the whole was extracted with a mixture of toluene and hexane (4/1). Combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate=9/1) to give 78 mg (91%) of a white solid. mp 64—65 °C. IR (Nujol) cm^{-1}: 1699, 1603. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (30H, m), 1.69 (2H, quintet, J=6.9 Hz), 3.83 (3H, s), 4.18 (2H, t, J=6.9 Hz), 6.31 (1H, d, J=15.9 Hz), 6.89 (2H, d, J=6.9 Hz), 7.47 (2H, d, J=6.9 Hz), 7.64 (2H, d, J=15.9 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 26.0, 28.7, 29.3—29.7 (29.28, 29.34, 29.52, 29.57, 29.63, 29.68), 31.9, 55.3, 64.5, 114.3, 115.8, 127.2, 129.6, 144.2, 161.3, 167.4. MS (EI) *m*/*z*: 430 (M^+), 178. HR-MS (EI) *m*/*z*: 430.3460 (Calcd for $C_{28}H_{46}O_3$: 430.3447).

(*E*)-1-(4-Hydroxyphenyl)docos-1-en-3-one (14) To a solution of 4-methoxymethoxybenzaldehyde (120 mg, 0.72 mmol) and henicosan-2-one (200 mg, 0.65 mmol) in a mixture of ethanol (10 ml) and THF (5 ml) was added an 8% aqueous solution of sodium hydroxide (0.2 ml), and the mixture was stirred for 5 d at rt. The reaction mixture was diluted with dichloromethane, and was washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate=9/1) to give 131 mg (33%) of (*E*)-1-(4-(methoxymethoxy)phenyl)docos-1-en-3-one as a white solid (53% calculated yield), together with unreacted ketone (32 mg, 16%). mp 67—68 °C. IR (Nujol) cm^{-1}: 1659, 1608. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.4 (32H, m), 1.67 (2H quintet, J=6.9 Hz), 2.63 (2H, t, J=6.9 Hz), 3.48 (3H, s), 5.20 (2H, s), 6.64 (1H, d, J=16.2 Hz), 7.05 (2H, d, J=6.9 Hz), 7.49 (2H, d, J=6.9 Hz), 7.51 (1H, d, J=16.2 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 24.5, 29.4—29.7 (29.35, 29.45, 29.49, 29.61, 29.68), 31.9, 40.9, 56.1, 94.2, 116.5, 124.6, 128.3, 129.8, 141.9, 159.0, 200.7. MS (EI) *m*/*z*: 458 (M^+), 206. HR-MS (EI) *m*/*z*: 458.3742 (Calcd for $C_{30}H_{50}O_3$: 458.3760).

A mixture of MOM-ether (55 mg, 0.12 mmol), concentrated hydrochloric acid (1 ml) in methanol (5 ml), THF (5 ml) and water (1.5 ml) was stirred for 24 h at rt. The reaction mixture was diluted with ethyl acetate, and was washed with water, saturated aqueous sodium bicarbonate, and brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate=4/1) to give 47 mg (94%) of **14** as a white solid. mp 96—97 °C. IR (Nujol) cm^{-1}: 3165, 1666, 1567. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.5 (32H, m), 1.68 (2H, quintet, J=7.2 Hz), 2.66 (2H, t, J=7.2 Hz), 6.63 (1H, d, J=16.2 Hz), 6.90 (2H, d, J=6.9 Hz), 7.01 (1H, br s), 7.44 (2H, d, J=6.9 Hz), 7.54 (1H, d, J=16.2 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 24.7, 29.3—29.7 (29.34, 29.43, 29.49, 29.69), 31.9, 40.8, 116.1, 123.6, 126.8, 130.3, 143.2, 158.6, 202.1. MS (EI) *m*/*z*: 414 (M^+), 162. HR-MS (EI) *m*/*z*: 414.3481 (Calcd for $C_{28}H_{46}O_2$: 414.3498).

DNA Polymerase Assays DNA polymerase α was purified from the calf thymus by immuno-affinity column chromatography as described by Tamai *et al.*[7] Recombinant rat DNA polymerase β was purified from *E. coli* JMp β5 as described by Date *et al.*[8] The activities of these mammalian DNA polymerases were measured by the methods described previously.[9,10] For DNA polymerases, poly(dA)/oligo(dT)$_{12-18}$ and 2′-thymidine 5′-triphosphate (dTTP) were used as template-primer DNA and nucleotide substrate, respectively. The synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) at various concentrations and sonicated for 30 s. Four microliters of the sonicated samples were mixed with 16 μl of each enzyme (final 0.05 units) in 50 mM Tris–HCl (pH 7.5) containing 1 mM dithiothreitol, 50% glycerol and 0.1 mM ethylenediaminetetraacetic acid (EDTA), and kept at 0 °C for 10 min. These inhibitor-enzyme mixtures (8 μl) were added to 16 μl of each of the enzyme standard reaction mixtures, and incubation was carried out at 37 °C for 60 min. The activity without the inhibitor was considered to be 100%, and the remaining activities at each concentration of inhibitor were determined as percentages of this value. One unit of each DNA polymerase activity was defined as the amount of enzyme that catalyzed the incorporation of 1 nmol of deoxyribonucleotide triphosphates (*i.e.*, dTTP) into the synthetic template-primers (*i.e.*, poly(dA)/oligo(dT)$_{12-18}$, A/T=2/1) in 60 min at 37 °C under the normal reaction conditions for each enzyme.[9,10]

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References and Notes

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